VALIDATION OF TEXAS BEEF JERKY PROCESSING

A Thesis

by

FELICIA DANIELLE ESPITIA

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2006

Major Subject: Animal Science

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Approved by:

Chair of Committee,	Jeff W. Savell
Committee Members,	Kerri B. Harris
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ABSTRACT

Validation of Texas Beef Jerky Processing. (August 2006)

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Chair of Advisory Committee: Dr. Jeff W. Savell

This study evaluated the thermal drying process commonly used by small and very small beef jerky operations in Texas. It was intended to determine the impact of relative humidity on the production of beef jerky and to provide documentation to beef jerky producers to support their Hazard Analysis and Critical Control Point programs. This project was divided into two phases: Phase I provided a low level of relative humidity (15-25%), whereas Phase II provided a high level (100%) for 25% of the cooking cycle. Both phases consisted of three trials, each representing one of the treatments (n=18) applied to the samples. The first treatment served as the control group and included samples that were non-inoculated, while the other two treatments included inoculations of samples with a bovine fecal slurry and rifampicin-resistant *Salmonella Typhimurium*. Each of the three treatments for both phases was analyzed for reduction of microbial levels in addition to temperature and product composition.

Once the two phases had been completed and all data were analyzed, it was concluded that there was not a statistical difference between the level of reduction for Aerobic Plate Counts, coliforms, *Escherichia coli* and *Salmonella* provided by Phase I with low humidity and Phase II with high humidity. Both levels of humidity provided

similar levels of reduction within each trial, suggesting that the level of humidity does not have a great impact on the level of microbial reduction achieved.

However, this study did not provide the adequate level of initial inoculation levels to support the required 6.5 log reduction stated in 9 CFR 318.7. Inoculation levels were lower than 6.5 logs for all three treatments in both phases, resulting in lower levels of overall reduction. Therefore, based upon the information provided by this study, it cannot be concluded that a low level of humidity will achieve a 6.5 log reduction as mandated in 9 CFR 318.17.

DEDICATION

I dedicate this to my parents, Noberto and Carmen Espitia. The love and support that they have given me has helped me in achieving my goals and becoming the person that I am today. I love you Mom and Dad!

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The funding for this project was provided by the Texas Beef Council and the National Cattleman's Association through the $1-per-head check off program made available by beef cattle producers.

I thank each of my committee members for participating in my undergraduate and graduate studies. Dr. Manuel Piña was very influential in my undergraduate studies by providing me the opportunity to learn about the international beef cattle industry. Dr. Gary Acuff was my undergraduate food microbiology professor who showed me that microbiology can be entertaining and fun to learn about. Dr. Kerri Harris has taught me an incredible amount about food safety and has helped me gain an interest for my future career. Dr. Jeff Savell has been very influential to me throughout my time as a student.

This research was completed with the help of several groups of people. Jason Bagley was always available to answer questions and assist me whenever needed. Lisa Lucia and the graduate students working in the lab were all very helpful to me with the lab work required for this project and taught me a great deal. Also, I thank Dr. Keeton for allowing me to use his lab and equipment to complete the composition analysis.

There were several graduate and undergraduate students who helped me in collecting data. Megan Laster, Bridget Baird, and Kristin Voges each provided a helping hand for me when asked. Finally, Kerri Bagley was always available and put in extra time in order to assist me in collecting data.

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INTRODUCTION

The production of jerky has become an important issue within the food industry as a result of various food borne outbreaks associated with its consumption. Although outbreaks have been documented as far back as 1966, it was an outbreak in October of 2003 that brought attention to the production of beef jerky and its safety to those who consume it. Epidemiological data suggested a relationship between the product produced by M.D. Chavez / Old Santa Fe Trail and several illnesses reported in New Mexico that proved to be associated with the exposure of *Salmonella*. As a result, a recall of 22,000 pounds of beef jerky was issued (FSIS, 2003).

With the evaluation of this case, as well as other documented cases, it has become evident that *Salmonella* is a concern in the production of beef jerky. It is important to determine that the current processes used in small and very small establishments to manufacture beef jerky are sufficient to eliminate the presence of *Salmonella,* as well as other microbiological hazards such as *Staphylococcus aureus* and *Escherichia coli* O157:H7, which are often associated with beef (FSIS, 2004). In June 1999, the Food Safety and Inspection Service (FSIS), an agency of the United States Department of Agriculture, released the latest edition of the Compliance Guideline for Meeting Lethality Performance Standards for Certain Meat and Poultry Products, which is referred to as Appendix A. This voluntary guideline, which producers are not required to follow, provided guidance to producers for meeting the lethality performance

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This thesis follows the style and format of *Meat Science*.

standards mandated in previous regulations for certain meat and poultry products (USDA, 1999). Unfortunately, numerous establishments within this industry are very small operations that do not have the resources available to validate their process. Due to this lack of resources, such as valid documentation, many smaller operators use Appendix A as support for their lethality step. However, by referencing this document, all of the requirements stated in this guideline must be met.

In March of 2004, FSIS released the Compliance Guideline for Meat and Poultry Jerky (Compliance Guideline for Jerky). This guideline was intended to provide updated information concerning the production of jerky and give further guidance to producers on how to alter their process in order to meet the performance standards previously set by FSIS in 9 CFR 318.17 of the Federal Regulation (FSIS, 2004). The new guideline emphasized the importance of the heating process and reinforced its potential to inactivate or kill any microorganisms that may be present. However, in order to ensure the quality of the product, it is vital that the process is done correctly and that the proper environment for lethality and drying is available. The FSIS Compliance Guideline for Jerky identifies the heating temperature and humidity level as the two factors that determine the lethality of the heating process.

With the release of this guideline comes the necessity for validating the ability to adequately control relative humidity and produce a safe product. Small and very small establishments that have relied only on the time/temperature requirements of Appendix A need additional scientific data to validate the lethality and drying processes normally used in most beef jerky operations.

The objectives of this study were: (1) to evaluate the thermal drying process commonly used by small and very small operations in order to validate the jerky production process, (2) to determine the impact of relative humidity on the production of beef jerky, and (3) to develop documents that could be used by small and very small beef jerky producers to support their Hazard Analysis and Critical Control Point (HACCP) programs.

REVIEW OF LITERATURE

The concept of drying meat with the application of smoke and heat has been practiced for centuries. The ancient Egyptians were one of the first civilizations credited with applying this concept for further preservation (FSIS, 2006). With time, other cultures began contributing to the development of the product as it is known today. For instance, the North American Indians dried fruit with meat and created a product referred to as “pemmican”. This drying process was originally the result of conservation to preserve large animals such as bear, buffalo, and whales, which were hunted and used for food, clothing, and shelter. Because these animals could not be entirely consumed at once, drying the leftover meat maximized the use of the animal. This was innovative and appealing to the nomadic lifestyle common for that time period (FSIS, 2006). During the period of the western settlement of the United States, the pioneer settlers contributed to the naming of this product. They used the Spanish word “charqui” as the basis for the word jerky in order to describe the dried meat product being made at that time (Nummer, Harrison, Harrison, Kendall, Sofos, & Andress, 2004). Today, marketing niches for jerky have been created making it a convenient product favored by hunters, backpackers, and those who simply enjoy the numerous types available.

Jerky is defined as “a nutrient-dense meat that is characterized as lightweight due to drying” (FSIS, 2006). Because of the lack of moisture, jerky is considered a shelf-stable product that does not need to be held at refrigerated temperatures or subjected to further processing in order to stay fresh (FSIS, 2006). Jerky is a unique product because

of its stability, exceptionally long shelf life, and convenience. It is often produced at the consumer level with a small dehydrator or warm oven, as well as commercially with the use of a large smokehouse. Commercially, beef is the most common choice for jerky although it is not exclusively limited to one species. Regardless of the species, muscles often chosen for production are lean cuts or are trimmed to remove excess fat, resulting in a lean product.

Jerky has been associated with at least nine reported food borne outbreaks since 1966 from both home dried and commercially manufactured products, which has brought attention to its safety as a food product (Nummer et al., 2004). In 1995, a major incident occurred that linked the illness of 93 persons who consumed jerky with *Salmonella* in New Mexico. This incident, coupled with the progressive implementation of the Hazard Analysis and Critical Control Point System from 1999 to 2000 for small and very small establishments, resulted in the amendment of federal meat and poultry regulations (Frey, 2004). Federal regulation 9 CFR 318.17 (a)(1) states that the production of cooked beef, roast beef, and cooked corned beef products need to achieve a 6.5 log reduction of *Salmonella*, or an alternative level of lethality, with an equivalent probability that the final product will be free of any viable *Salmonella* organisms (USDA, 1996c & 1999). FSIS released Appendix A that provided the necessary time that a product should be held at various internal temperatures in order for the 6.5 log reduction to be met and further discussed the need for applying wet heat during the cooking cycle (USDA, 1999).

The parameters described in Appendix A were the result of research published by

Goodfellow and Brown (1978). This research was in response to USDA's consideration of amending the regulation that required establishments processing cooked beef and beef roast products to reach a minimum internal temperature of 63 °C. USDA requested research to introduce revised data on D-values for *Salmonella* serotypes within a meat system, in addition to sound time-temperature processes that would ensure a proper eradication of *Salmonella* when present on wet or steam cooked and dry roasted beef. Before the publication of this research, beef systems had not been analyzed to adequately determine D-values, and proper time-temperature processes had not been established for the production of *Salmonella* free "rare" roast beef. It was the objective of the Goodfellow and Brown (1978) study to determine these values and established proper time-temperature processes for "rare" roast beef.

The information obtained from the publication of this study allowed for the construction of processing schedules that listed internal temperatures with the corresponding process times needed to adequately eliminate *Salmonella*. Furthermore, it was clearly proven that the use of wet heat drastically reduced the amount of time necessary to inactivate any *Salmonella*. However, dry heat could only eliminate *Salmonella* from the surface of a dry roasted product greater than 10 pounds having a minimum internal temperature of 54 °C, and held in an oven with an ambient temperature of 121 °C (Goodfellow & Brown, 1978).

In October of 2003, a New Mexico jerky producer voluntarily recalled 22,000 pounds of beef jerky because of possible contamination with *Salmonella* (FSIS, 2003). This event prompted the release of the 2004 Compliance Guideline for Meat and Poultry

Jerky. As previously stated, this guideline was intended as a reference to jerky producers. It describes each step within the process and discusses their role in providing a safe product. Although each producer's process may vary, the main steps commonly applied in jerky production are discussed in the compliance guideline. These include strip preparation, marination, interventions, applying a lethality treatment, drying, and handling (FSIS, 2004).

Preparing the strips is the quickest and easiest of all the steps. Whole muscles are sliced to a preferred thickness and are usually cut in the same direction as the muscle fibers in order to enhance the quality. If slicing is not preferred, an alternative is to grind the product and manually form the strips (FSIS, 2004). Grinding does, however, present a greater risk for contamination. This is due to the increase in product handling as well as the use of equipment that can disseminate any pathogens that may be present throughout the final product (Faith, Le Coutour, Bonnet, Alvarenga, Calicioglu, Buege, & Luchansky, 1998).

Marination is an optional step, however, it is commonly practiced because it enhances flavor. The strips can be placed into a solution that may contain ingredients such as salt, soy sauce, sugar, sodium nitrite or any other flavor enhancers depending upon the type of product being produced. The amount of time the strips will remain in the solution will vary with each operation (FSIS, 2004).

An intervention step is optional and may be put into place to further enhance the lethality step. Some interventions suggested in the guideline include heating the meat to a minimum temperature of 71 °C while in the marinade, or applying an acid dip before

placing the strips in the marinade (FSIS, 2004). Calicioglu, Sofos, Kendall, and Smith (2002) concluded that pre-drying treatments, including various combinations of acetic dips, reduced the viability of *Salmonella* when contaminated after processing. Although an intervention step has been shown to further improve the level of pathogen reduction, it is not intended to replace the following lethality step (FSIS, 2004).

A proper lethality treatment must be applied in order to eliminate the presence of any pathogens and guarantee a safe product. Based upon previous research from Goepfert, Iskander, and Amundson (1970), it is essential that this step is adequately executed and the proper environment is provided (FSIS, 2004). Goepfert et al. (1970) concluded that a lower a_w level would result in heat resistant cells in *Salmonella*. This information provided support for the humidity requirements provided in this document.

Once the lethality treatment has been applied, the product will need to be dried to an acceptable level to guarantee the proper surface conditions are maintained. It is important that this process be performed correctly to ensure that the product is dry and all bacterial pathogens that may be present are inactivated (FSIS, 2004).

Finally, the importance of product handling is discussed within the compliance guideline. Once the jerky product has received the proper lethality and drying treatments, the impact of post treatment adulteration is much greater. This is because the product will not be refrigerated or heated, eliminating any further interventions that may control, reduce, or eliminate any biological hazards (FSIS, 2004).

Most commercial operations are considered to be small and very small limiting their resources to meet federal regulations and guidelines. Therefore, a producer must

have a thorough HACCP system that is properly designed and executed. The Pathogen Reduction/HACCP rule was introduced in July of 1996 and required implementation in 1998, 1999, and 2000 for large, small, and very small establishments, respectively (FSIS, 1997). HACCP was designed as a preventive system that identifies potential biological, chemical, and physical hazards within a process (USDA, 1996b). The biological hazards most commonly associated with jerky have been various strains of *Salmonella sp*. as well as *Staphylococcus aureus* (Eidson, Sewell, Graves, & Olson, 2000). *Escherichia coli* O157:H7 also has been identified as a potential hazard because of its relationship with meat products. Since its first identification in 1982, *E. coli* O157:H7 has been associated with 73,500 food borne illnesses, with 1,800 hospitalizations and 50 deaths annually within the United States. However, because it is most commonly related to raw products (Murphy, Martin, Duncan, Beard, & Marcy, 2004), it is considered a minimal risk for a product subjected to further processing.

Salmonella is of greatest concern for the jerky industry as a result of its past relationship with this product. The genus *Salmonella* is a member of the family *Enterobacteriaceae* and is characterized as gram negative, non-spore forming rods that are facultatively anaerobic, oxidase negative, and glucose fermentative. Movement is achieved with the use of peritrichous flagella that are present over the entire surface of the body. About 2,400 serotypes have been identified for *Salmonella sp*. This is based upon the Kauffman-White serotyping scheme used to differentiate within a genus based upon their somatic (O) and flagellar (H) antigens (Jay, 1998).

The disease caused by *Salmonella sp.* is termed Salmonellosis, which is defined

as a zoonotic disease because infected animals are the source of contamination for human illness. Infection is generally through a fecal to oral route of contamination, which is typically accomplished through the consumption of food or water (Jay, 1998). Symptoms most often appear suddenly and may include nausea, abdominal cramps, vomiting and diarrhea. Additional physical signs may involve muscular weakness, feeling faint, moderate fever, restlessness, twitching and drowsiness which may occur after the initial onset of Salmonellosis (Frazier, 1967). Within the United States in any given year, 40,000 cases of Salmonellosis are reported annually with about 1,000 of those resulting in death (Murphy et al., 2004). Several factors have been known to influence the severity associated with an infection. One major factor is the susceptibility of the individual involved. Any person with a compromised immune system is more likely to develop more severe symptoms or death (Center for Disease Control, 2005). Additionally, the particular strain of *Salmonella* as well as the level of infection can greatly influence the onset of the disease and how it affects the consumer (Frazier, 1967).

Environmental conditions necessary for optimal growth are a temperature of 37 °C, a pH of 7.0, and a minimum water activity level of 0.93. Nevertheless, *Salmonella sp.* is able to maintain a steady growth in conditions with broader parameters. For instance, growth has been reported with temperatures ranging from 5 to 47 °C and in low-acidic foods (Jay, 1998).

Within a facility's HACCP plan, critical control points (CCP) are identified as steps within the process that control, reduce or eliminate the potential hazard identified

for that product. In the absence of a CCP, the hazard identified could pose as a food safety risk for the final product. The CCP identified for most jerky operations is cooking. This step provides the adequate amount of heat and time needed to sufficiently neutralize any pathogen that may potentially be present. With the absence of this step, any pathogen present on the raw product would be expected on the finished product (National Advisory Committee on Microbiological Criteria for Foods, 1992).

Complete drying of the product is also important for producers to address in order to ensure that the moisture-to-protein ratio (MPR) achieves a level of 0.75:1. This is the accepted standard that identifies a product as jerky. Traditionally, the MPR was used by producers to determine if the proper level of drying had been achieved. However, it has been determined that this is not an adequate interpretation to the level of water activity (a_w) present on the product. Therefore, producers need to consider the water activity level of their product. Jerky products require a minimum water activity level that is equal to or less than 0.85, the standard measurement for growth of *S. aureus* (FSIS, 2004).

To date, various research projects have been designed to evaluate different aspects of jerky. Some have established a relationship between this product and various pathogens that may be of concern. Others have assessed pre-processing treatments and their effect on the viability of various organisms during post-processing drying and storage. Even further discussions have focused on how these treatments may influence consumer acceptability. With the publication of each study comes a greater understanding for this product and what is necessary to produce a safe jerky product.

Holley (1985a,b) evaluated the influence of jerky manufacturing and storage on the viability of certain pathogenic microorganisms. Holley (1985a) focused specifically on the behavior of *S. aureus* when present in high numbers and subjected to a home dehydrator. It was not designed to represent levels that would be expected in most commercial operations. Meat samples were taken from raw inside round and corned beef brisket. After applying a 4 h drying period of 52.9 $\pm$ 0.8 °C, followed by an additional 4 h cycle at 48.2 $\pm$ 0.4 °C, some important observations were made. First, the temperatures used in this study were the result of temperature discrepancies from the equipment used. Actual temperatures were set at 68.3 °C and 60 °C, respectively. It was determined that the temperatures obtained during the study would be used to represent most in-home operations.

The initial drying of the product provided an immediate decrease in the number of coliforms, however, within 2 h, an increase in spore formers was observed in the inoculated slices for both samples. Uninoculated corned beef slices only showed an increase after 4 h. An 8 h drying period caused a greater decrease with half of the organisms recovered on the inside round slices and 75% present on the corned beef brisket identified as non-recoverable. It was noted that this process did destroy coliforms that were viable along with naturally occurring staphylococci present.

Holley (1985a) identified the most perilous stage in the jerky production process as the initial drying period when the level of a_w was above 0.86. *S. aureus* can grow at a lower a_w than 0.86 while other pathogenic organisms do not. Thus, it is imperative that jerky be dried to a level lower than 0.86 within a specified amount of time. Samples

were stored in refrigerated temperatures for a period of 8-9 days in moisture-permeable bags. It was noted that a significant reduction also was obtained from both the total number of viable coliforms as well as inoculated staphylococci when in this environment. Some concerns discussed were the possibility of commercially cured meats maintaining a higher a_w level for a longer period of time, especially if processed in a dehydrator that was operating at full capacity. These two observations, which are common throughout jerky processing, could inhibit the product from maintaining an adequate moisture level in the environment within the specified time needed.

Holley (1985b) reported research designed to provide answers for questions left open by the previous study. This paper sought to determine if the increase in total aerobic bacteria and staphylococci were accurate and could be expected in a second study. Of concern was the impact of providing available space within the dehydrator when processing jerky, because this could alter the level of pathogen reduction and, to determine if these organisms could flourish under similar conditions, but at lower inoculation levels. Not only did this paper address these issues, it also evaluated other food pathogens of concern to broaden the application of the data.

Holley (1985b) determined that staphylococci present in high numbers did provide the potential for a significant increase in total number within the initial 2 h period of drying. Furthermore, it was found that the arrangement of product within the dehydrator had an impact on drying rate. A fully loaded dehydrator reached the desired a_w level of 0.86 between 2.5 – 3 h. This was compared to a dehydrator that was half-full which only required 1 – 2.5 h to reach this same level. The information obtained from

this study allowed for the final determination that in-home product could be safely produced with a dehydrator as long as the desired temperatures were met within the appropriate time frame.

The effects of pre-drying marinade treatments have been studied to determine their level of influence on the behavior of pathogens after the product has been processed. Two studies with similar designs (Calicioglu et al., 2002; Calicioglu, Sofos, Samelis, Kendall, & Smith, 2003) have observed *Salmonella* in these conditions. Various marinade solutions were applied to determine their efficacy when comparing acid-adapted and non-adapted strains of *Salmonella*. Calicioglu et al. (2002) concluded that the pre-drying treatment and the characteristics associated with the culture used for inoculation have a significant impact on the behavior of *Salmonella* present after processing. It was determined that *Salmonella* cultures that were acid-adapted and present after processing were notably more sensitive than the non-adapted cells. Furthermore, Calicioglu et al. (2003) obtained results that were similar to the data published one year prior. They found that acid-adapted cells of *Salmonella* were no more resistant than that of non-adapted cells. Both studies concluded that the use of certain food grade chemicals combined within the marinade provided the best results.

An undesired consequence of various pre-processing treatments implemented to enhance the level of pathogen reduction and guarantee a safe product can alter overall jerky quality and jeopardize product acceptability by the consumer. Therefore, it is essential to study the effects of these treatments on the overall quality to guarantee that the characteristics favored by consumers are maintained and not corrupted by the

process. Harrison, Harrison, Rose-Morrow, and Shewfelt (2000) evaluated four methods of jerky preparation to determine their effect on overall quality in the final product. These four methods included: a traditional overnight marination and dehydration at 60 °C; a treatment that heats the samples in an oven at 135 °C for 10 min; a treatment to eliminate the dehydration step and instead boil the marinade and jerky pieces for about 10 min prior to dehydrating at 60 °C. Treatment 4 marinated the strips overnight, heated them in an oven at 163 °C for 10 min and then followed with a dehydration step at 60 °C. All four treatments were accomplished with marinade as well as without marinade. Various factors were analyzed such as the microbial activity of *E. coli* O157:H7, *Listeria monocytogenes*, and *Salmonella*, in addition to texture, color, off-flavors, saltiness, and overall consumer acceptability.

From the methods listed, it became apparent that the application of a marinade coupled with an additional heating process could further enhance the level of lethality. However, descriptive attribute evaluations such as color, texture, and saltiness observed with Treatments 3 and 4 yielded lower scores for texture and saltiness and averaged higher on texture. Furthermore, overall consumer acceptability was determined to be lowest for Treatment 4 whereas, Treatment 2 and 3 were not significantly different from Treatment 1.

Albright, Kendall, Avens, and Sofos (2003) evaluated pre-drying treatments and their effect on the inactivation of *E. coli* O157:H7 to achieve a 5-log reduction when inoculated onto beef jerky. This study used a multiple-hurdle concept, which enables individual factors to be applied at lower intensities when combined with other factors.

The authors identified the most important hurdles for their research as temperature, water activity (a_w), acidity (pH), redox potential (E_h) and preservatives (organic acids, spices). The four pre-drying treatments included 1) immersing in boiling water (94 °C) for 15 s followed by marinating (4 °C) for 24 h; 2) seasoning with pickle spices (4 °C) for 24 h followed by an immersion in hot pickle brine (78 °C) for 90 s; 3) immersing in a warm vinegar/water solution (57 °C) for 20 s followed by marination (4 °C) for 24 h; and 4) and marinating (4 °C) for 24 h followed by immersing in a warm vinegar/water (57 ° C) solution for 20 s.

The initial 4 h drying period proved successful in achieving a significant destruction for all treatments. Although levels continued to decline during the 10 h drying period, a 5-log reduction was achieved only by applying a seasoning with pickle spices followed by an immersion in hot pickling brine. Applying a boiling water-marinade treatment was not recommended by the authors as it did not achieve a 5 log reduction and resulted in a high water activity level of 0.75. *E.* coli O157:H7 levels did decline during an initial application of boiling water; however, it was hypothesized that the marinade may have provided a desired environment needed for the inoculated cells to recover and multiply.

Additional research conducted by Yoon, Calicioglu, Kendall, Smith, and Sofos (2004) further demonstrated the behavior of *E. coli* O157:H7 during the production of jerky. Samples were inoculated with low (4 log cfu/cm^2) and high (7 log cfu/cm^2) levels of *E. coli* O157:H7 and were subjected to no treatment, traditional marinade or a 5% acetic acid solution which was followed by a traditional marinade. Inactivation levels

during drying at 60 °C for 10 h and storage at 25 °C for 60 days were evaluated. It was concluded that the 5% acetic acid combined with a traditional marinade provided the greatest level of reduction. Observations from this study suggest that the application of 5% acetic acid may help sensitize the cells at higher inoculation levels resulting in a more effective inactivation when dried, further enhancing the overall destruction of cells.

From the preceding articles, some information has been provided to jerky processors to assist in the production of a safe and high quality product. However, further research is needed to confirm the effectiveness of other processing parameters and to enhance the value of this product.

MATERIALS AND METHODS

Jerky Processing

Beef inside rounds, cap off (IMPS #169A; NAMP, 1997; USDA, 1996b) were obtained from the Rosenthal Meat Science and Technology Center (RMSTC) at Texas A&M University. The rounds were trimmed free of external fat and connective tissue and frozen at –23 $\pm$ 2 °C. After freezing, whole muscles were sliced with the grain of the muscle fibers on an electric band saw into 6.35-mm thick slices. Slices of whole inside round muscles were placed into plastic (Rubbermaid®, Newell Rubbermaid, Inc., Freeport, IL) barrels capable of holding 167 L. These barrels contained a brine solution consisting of 37.85 L of water, 4.12 kg of a commercial seasoning (Reo Jerky Seasoning, Reo, Inc., Huntsville, TX) consisting of salt, sugar, and hickory smoke flavor, and 113.40 g of Prague powder (Heller's Modern Cure, Heller Seasonings, Chicago, IL) per 45.36 kg of meat. Slices were stored in this brine solution for a period of seven to seventeen days before being hung onto rods and placed into an Alkar Model 1003 smokehouse and drying chamber (Alkar, Lodi, WI). The number of days that samples were stored in the brine was dependent on the production schedule for this project.

Experimental Design

Rosenthal Center is a very small, state inspected establishment that uses the same basic equipment and processes as other small and very small plants across Texas. Relative humidity of the smokehouse was determined by using the differential between

dry bulb and wet bulb temperature thermometers inside an Alkar smokehouse, and the relative humidity was predicted from a psychometric chart. For the production of most jerky, the wet bulb temperature is usually at 17.8 °C and dry bulb temperature is between 60 and 93.3 °C; therefore, the relative humidity is negligible. Based upon Appendix A (USDA, 1999), the relative humidity should be at least 90% for 25% of the cooking process, providing it is not less than one hour; however, most jerky is not heated in this manner. To compare the effects of low humidity and high humidity heating, this project was divided into two phases. The first phase used normal jerky processing procedures that focused on time and temperature with a very low relative humidity. During the second phase, the relative humidity was increased to 90% or greater to determine the impact of the relative humidity on the quality characteristics of the jerky. Both phases consisted of trials designated to evaluate microbial counts for a rifampicin-resistant *Salmonella* Typhimurium, as well as *E. coli*, coliforms, and Aerobic Plate Counts (APCs). In addition, moisture, protein, and water activity were measured. Both phases involved three trials. The first trial consisted of non-inoculated samples (n=18) and served as a control group. This trial represents traditional product sold from the Rosenthal Center and was repeated three times for each phase.

For the second trial, a bovine fecal slurry was prepared for inoculating samples by placing 200 g of bovine feces and 1800 ml of 0.1 % sterile peptone water (Difco Laboratories, Detroit, MI) into an autoclave tub, where the slurry was stirred. The inoculation was completed by dipping the samples (n=18) into a fecal slurry for 30 s. The samples were removed and allowed to stand 30 m to allow the slurry to completely

inoculate the sample. After the dwell time, the inoculated samples were placed in bags and sealed, and then placed into a second bag and sealed to comply with laboratory protocol before transportation to the Rosenthal Center for processing. The slices of inoculated beef remained in the sealed bags until being placed in racks inside a smokehouse and drying chamber. All opened bags used for the beef slices, disposable protective clothing, and gloves were collected in a biohazard bag immediately after placing the samples in the smokehouse. The contaminated materials were transported to the Food Microbiology Laboratory for sterilization and disposal. This trial was repeated three times for each phase.

The third trial involved inoculation of the beef strips with *S.* Typhimurium. The samples (n=18) were inoculated with a rifampicin-resistant strain of *S.* Typhimurium in the Food Microbiology Laboratory. The inoculation was completed by dipping the samples into a 10^8 log colony forming unit (CFU/ml) suspension of rifampicin-resistant *S.* Typhimurium for 30 s. The samples were removed and allowed to stand 30 m. After the dwell time, the samples followed the same procedure described above for transportation to the Rosenthal Center for processing. Once the slices of inoculated beef were placed inside the smokehouse, all bags, protective clothing, and gloves were sterilized and disposed of in the Food Microbiology Laboratory. This trial included three repetitions.

Once the heating and drying process was completed, the samples were removed from the smokehouse and immediately placed into unused, sterile bags that were sealed for transportation back to the Food Microbiology Lab for post processing analysis. The

racks and trucks were placed into the smokehouses and heated at 93.3 °C for a minimum of 5 h to sterilize the racks, trucks, and oven.

Microbial Analysis

Jerky samples were tested in the Food Microbiology Laboratory for aerobic plate counts, *E. coli,* coliforms, and rifampicin-resistant *S.* Typhimurium before and after thermal processing for each replication. A 10-cm^2 section of jerky (n=18) was aseptically removed and placed into a sterile bag containing 99 ml of 0.1% sterile peptone water. A Tekmar 400 Lab Blender Stomacher (Tekmar, Cincinnati, OH) was used to pummel the sample for 1 m. Serial dilutions of the homogenate were plated onto Petrifilm™ Aerobic Plate Count (APC) Plates and Petrifilm™ *Escherichia coli*/Coliform Count Plates (ECC) (3M Microbiology, St, Paul, MN). APC plates were incubated at 25 °C for 48 h. All colonies were counted and reported as log CFU/cm^2. ECC plates were incubated at 37 °C for 48 h and all *E. coli* and coliforms were counted and reported as log CFU/cm^2.

Counts for marker microorganisms were determined by plating onto pre-poured and dried rifampicin-tryptic soy agar (rif-TSA) plates (Difco). Rif-TSA was prepared by adding a solution of 0.1 g of rifampicin (Sigma, St. Louis, MO) dissolved in 5 ml methanol (EM Science, Gibbstown, NJ) and diluting to 1 L of autoclaved and cooled (55 °C) TSA (Difco). Rif-TSA plates were incubated for 24 h at 37° C before counting and reported as log CFU/cm^2.

Temperature Analysis

Ambient temperatures were recorded throughout the entire processing cycle with

the use of a strobe located inside the smokehouse. Additionally, surface temperatures were determined at specific times with the use of an infrared thermometer (DeltaTRAK, Inc., Pleasanton, CA). Initial temperatures were recorded before the samples were placed into the smokehouse, as well as at 0, 5, and 15 m after completing the heating and drying process.

Relative Humidity

The level of relative humidity was determined by recording the wet and dry bulb temperatures of two thermometers located inside the smokehouse. One thermometer was covered with a wetted sock while the other was maintained dry. The wet and dry bulb temperature differential readings were obtained from a psychometric chart and relative humidity was determined at differential stages of heating. These readings were recorded on the smokehouse temperature charts which are used during normal beef jerky processing at RMSTC.

Composition Analysis

Samples from all three trials within both phases (n=42) were analyzed for water activity, moisture, and protein. Water activity levels for each sample were determined on the same day as removal from the smokehouse. Each slice of jerky was blended in a food processor located in an additional laboratory at Texas A&M University. Samples were measured based upon the protocol established by the manufacturers of the Aqualab Water Activity Meter – Series 3 (Decagon Devices, Inc., Pullman, WA), which was used to determine the level of water activity for each sample. Triplicates were run from each sample in order to identify any variation within each sample.

After the a_w was read, all samples were frozen for several weeks before completing the moisture and protein analysis. These analyses were completed to obtain the moisture-to- protein ratio and confirm that the product produced met the 0.75:1 moisture-to-protein ratio specified for jerky. Moisture was determined using a forced-air connection oven drying method. Three grams of each sample were placed into a dried paper thimble and weighed before to being placed in the oven. The samples were dried in the oven for a minimum of eighteen hours. Once dry, they were removed, allowed to cool, and reweighed to determine the difference and obtain an overall moisture level. This analysis also was performed in triplicates to account for sample variation.

The percent protein present of each sample was determined with the use of a LECO nitrogen analyzer (LECO Corporation, St. Joseph, MI). Three grams from each sample were placed into the LECO nitrogen analyzer, combusted and the percent nitrogen was multiplied by 6.25 to obtain the percent protein. Triplicates of each sample were analyzed.

Statistical Analysis

Data collected from both phases were analyzed using SAS (SAS Institute, Cary, NC). Simple statistics were generated with the PROC MEANS procedure to complete the temperature and composition analysis. The microbial analysis was accomplished with PROC GLM to test for significance ($P< 0.05$) by analysis of variance.

RESULTS AND DISCUSSION

During completion of this research, modifications were made to the original experimental design. Samples from all three trials within Phase 1 (n=63) post-treatment were analyzed only for microbial data because of a concern that inoculated samples would possibly contaminate other laboratories. After the completion of Phase 1 and prior to Phase 2, it was decided to choose seven samples from each rep (n=21) that were non-inoculated and placed into the smokehouse with the intention of providing information on the product composition. Therefore, to obtain information on samples subjected to the environment of Phase 1, additional samples (n=21) were prepared with the same conditions as described above and were cooked using the low humidity parameters.

Microbial Data

Microbial data from both Phase 1 and Phase II are presented in Table 1. APC counts showed a reduction of at least 3.1 and 1.7 $\log_{10}$ CFU/cm^2 for the non-inoculated and bovine slurry samples during Phase I, respectively. Similarly, Phase II demonstrated a reduction of at least 2.9 and 1.1 $\log_{10}$ CFU/cm^2 for both of these treatments suggesting a relationship between the two phases.

E. coli levels for the non-inoculated samples in Phase I were at undetectable levels initially and therefore remained at this level after treatment. Bovine slurry samples were reduced by at least and 2.6 $\log_{10}$ CFU/cm^2. In Phase II, non-inoculated samples remained constant at undetectable levels whereas samples inoculated with the bovine slurry were reduced by at least 1.2 $\log_{10}$ CFU/cm^2. Yoon et al. (2004)

demonstrated the behavior of *E. coli* O157:H7 during the production of jerky. When comparing untreated samples to those with a traditional marinade and a marinade combined with acetic acid, *E. coli* O157:H7 reacted more to the presence of acetic acid. Harrison et al. (2000) also tested the effect of marinades on the presence of *E. coli* and found that a traditional marinade resulted in a 5.8 log reduction when samples were dried at 60 °C. This level of lethality was increased even further when salt was added to the marinade solution. Additionally, Faith et al. (1998) studied the viability of *E.* coli O157:H7 on jerky prepared at levels of 5 and 20% fat. Time and temperature relationships were determined to achieve a 5-log reduction for both fat levels. Overall, samples with a 5% fat content reached this desired reduction level in less time than those with 20% fat, which suggests that jerky slices prepared with lower fat contents may provide additional antimicrobial properties. Although these studies evaluated different aspects of jerky processing, the relationship between pre-drying treatments such as marinades and product composition, such as fat content, can be established in relation to expected inactivation levels.

Furthermore, non-inoculated coliforms remained constant at undetectable levels for both phases whereas the bovine slurry saw a reduction of at least 2.8 and 1.3 $\log_{10}$ CFU/cm^2 for Phase I and Phase II, respectively.

Salmonella levels (Table 2) were reduced by 4.8 and 4.9 $\log_{10}$ CFU/cm^2 to undetectable levels during Phase I and Phase II, respectively. Goodfellow and Brown (1978) reported a similar relationship when comparing dry and steam injected processes for roasted beef. *Salmonella* was reduced to undetectable levels in both oven roasted

beef held at an internal temperature of 51.7 °C with both the dry and steam injected process requiring similar cooking times. Additionally, Harrison et al. (2000) studied the reduction levels of *Salmonella* when marinades were used in combination with various cooking cycles. This study found that a traditional marinade could provide a 4.6 log reduction when samples were held at 60 °C. With the addition of a salt-containing marinade, this level of reduction was increased even further.

Temperature Analysis

Table 3 presents both initial and final temperatures for both phases. Initial temperatures were recorded prior to the samples being placed into the smokehouse with an average temperature of 18.4 and 18.2 °C for both Phase 1 and Phase 2. Final temperatures also were recorded at 0, 5, and 15 min after the samples had completed the heating and drying process. The mean temperatures were 45.0, 38.4 and 35.1 °C for Phase1 whereas Phase 2 had an average of 49, 42.5 and 36.4 °C for 0, 5 and 15 min, respectively.

While completing this research, several constraints became obvious. First, recording the final surface temperatures was difficult to obtain due to the rapid dissipation of heat once the door to the smokehouse was opened. Also, determining an internal temperature of the product proved to be challenging as the samples were very thin. The probe located in the smokehouse was larger in diameter and needed to be strategically placed within the sample for it to remain throughout the entire time the product was in the smokehouse.

Relative Humidity

Phase I was designed to illustrate the process used commonly by small and very small establishments that produce jerky. The cooking cycle used for this phase consisted of three steps each consisting of 30 min, 15 min and 3 h. The blowers were on during this time while the damper was on only for the final 3 h. This was followed by a drying cycle that lasted 12 h and had only the blowers on. The average relative humidity for the first 30 min was 18 %; the next 15 min provided 17% followed by 3 h with 21%. The 12 h drying cycle was 15% relative humidity for all three trials.

Phase II was modified to satisfy the Compliance Guideline for Jerky. During the initial cooking cycle, the relative humidity was 100% for the first hour. Once this level was met for the specified time, conditions were returned to the normal processing environment as stated above.

Composition Analysis

Data from the composition analysis are presented in Table 4. Water activity levels were determined for both phases with a level of 0.5 for Phase 1 and 0.6 for Phase 2. Moisture and protein levels were 13.8 and 64.1 for Phase 1, and Phase 2 obtained levels of 17.9 and 63.5, respectively. This resulted in moisture-to-protein ratios of 0.23:1 for Phase 1 and 0.28:1 for Phase 2. Both phases provided the adequate ratio necessary to identify the product as jerky.

CONCLUSION

Upon evaluation of both the microbial and composition characteristics of the samples subjected to the low versus high humidity levels, conclusions were made based upon the objectives outlined for this study. The procedure used at Rosenthal Center to produce beef jerky was proven to be sufficient in reducing the level of *Salmonella* even when low levels of humidity were present during the lethality step. When compared to Phase II with the high humidity for at least 25% of the cooking time, the reduction levels were statistically similar. It became apparent that there were not any significant differences between the two treatments as both levels of humidity provided reductions to undetectable levels. Furthermore, under these conditions, it is apparent that the level of relative humidity applied during the cooking cycle does not greatly influence the level of pathogen reduction in this jerky production system.

However, it cannot be concluded that this research obtained the required 6.5 log reduction as required by 9 CFR 318.17. Therefore, the data obtained from this study cannot be used as a validation resource for small and very small beef jerky producers. Further research with initial inoculation levels higher than those required by the regulations is needed in order for an accurate estimation of lethality to be determined.

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APPENDIX A

TABLE 1

Least squares means (n=54) of $\log_{10}$ (CFU/cm^2) and p-values of APC, *E. coli*, and coliforms during Phase 1 and Phase 2.

	APC			*E.coli*			Coliforms		
	Initial	Final	P-Value	Initial	Final	P-Value	Initial	Final	P-Value
Phase 1									
Non-Inoculated	4.1	<1.0	<.0001	<1.0	<1.0	<.0001	<1.0	<1.0	0
Bovine Slurry	4.7	3.0	<.0001	3.6	<1.0	<.0001	3.8	<1.0	<.0001
Phase 2									
Non-Inoculated	3.9	<1.0	<.0001	<1.0	<1.0	<.0007	<1.0	<1.0	0
Bovine Slurry	4.2	3.1	<.0001	2.2	<1.0	<.0001	2.3	<1.0	<.0001

Table 2

Least squares means (n=54) of log_{10} (CFU/cm^2) and p-values of *Salmonella* levels during Phase 1 and Phase 2.

	Salmonella		
	Initial	Final	P-Value
Phase 1			
Salmonella	5.5	<1.0	<.0001
Phase 2			
Salmonella	5.6	<1.0	<.0001

TABLE 3

Means (n=54) and standard deviations for initial and final temperatures for Phase 1 and Phase 2.

		Mean		Standard Deviation	
		Phase 1	Phase 2	Phase 1	Phase 2
Initial Temperature (ºC)		18.4	18.2	5.4	5.1
Final Temperature (ºC)					
	0 min	45.0	49.0	10.9	9.9
	5 min	38.4	42.8	7.9	8.2
	15 min	35.1	36.4	4.7	5.5

TABLE 4

Means (n=54) and standard deviations for water activity (a_w), moisture and protein for Phase 1 and Phase 2.

	Mean		Standard Deviation	
	Phase 1	Phase 2	Phase 1	Phase 2
Water Activity (a_w)	0.5	0.6	0.02	0.03
Moisture	13.8	17.8	1.2	1.7
Protein	64.1	63.5	2.2	7.4
Moisture-to-Protein Ration	.23:1	.28:1	0.03	0.03

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